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THE CHEMISTRY OF TYROTOXICON:

ITS ACTION UPON LOWER ANIMALS; AND ITS RELATION TO THE SUMMER DIARRHEAS OF INFANCY.

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Since making my last report to this Board on the investigations concerning the nature of tyrotoxicon (Report of Proceedings of the Michigan State Board of Health, October, 1886), I have continued my work, aided greatly by Messrs. F. G. Novy and E. V. Riker. We soon ascertained that if some butyric acid ferment be prepared as is ordinarily done in the preparation of butyric acid, and some of this be added to normal milk, and the whole be kept in closely stoppered bottles for eight or ten days, the poison will be developed in the milk in considerable quantity. The milk should be filtered, the filtrate neutralized with sodium carbonate, and then extracted with ether.

Having a strong solution of the poison in absolute alcohol, which had been obtained from milk inoculated as stated above, we added to it some platinum chloride and began to evaporate on the water-bath. As soon as the alcohol evaporated, the residue exploded with great violence. The vessel, a glass evaporating dish, was broken into fine fragments, and these were scattered over the room; while the gas-light of burner under the water-bath was extinguished. The experiment was repeated a number of times with like results. From some of this alcoholic solution, the platinum was removed with hydrogen sulphide gas; but the filtrate was then found to have lost its explosive property. This reminded us that diazobenzol compounds form with platinum chloride a highly explosive compound, and that these are also decomposed by hydrogen sulphide. Some diazobenzol nitrate was prepared according to the method of Griess,* and comparisons made between this and tyrotoxicon.

With equal parts of sulphuric acid and carbolic acid the prepared diazobenzol nitrate gave a green coloration; while with the same reagents,

*Annalen der Chemie und Pharmacie, B. 137, S. 39 et seq.

tyrotoxicon gave a color which varied from a yellow to an orange-red. But the diazobenzol nitrate dissolved in the whey of normal milk, and extracted with ether or in the presence of other proteids, gave the same shades of color as the tyrotoxicon did, and, the potassium compound of tyrotoxicon, prepared by the method to be given later, produced the same shade of green as did the artificial diazobenzol. This color test may be used as a preliminary test in examining milk for tyrotoxicon. It is best carried out as follows: place on a clean porcelain surface two or three drops each of pure sulphuric acid and pure carbolic acid. This mixture should remain colorless or nearly so. Then add a few drops of the aqueous solution of the residue left after the spontaneous evaporation of the ether. If tyrotoxicon be present, a yellow to an orange-red color will be produced. This test is to be regarded as only a preliminary one; for it may be due to the presence of a nitrate or nitrite.* The tyrotoxicon must be purified according to a method to be given further on, before the absence of nitrate or nitrite can be positively demonstrated.

In the filtrate from milk which is rich in tyrotoxicon, after neutralization with sodium carbonate, filtration and acidifying with hydrochloric acid, gold chloride produces a precipitate, which is insoluble in water, but soluble in hot alcohol, from which it separates on cooling in golden plates. Diazobenzol compounds give with gold chloride a precipitate having all these properties. In both cases the gold compound is decomposed by frequent treatment with hot alcohol, and this fact prevented any satisfactory ultimate analysis of this salt. It should be remarked here that from some samples of milk this gold salt is obtained much more easily than from others, and the difference is dependent not so much upon the amount of tyrotoxicon present, as upon the condition of the other organic matter present. It is best obtained from samples which have stood in well stoppered bottles for a month or longer.

Tyrotoxicon obtained from milk was treated according to the method recommended by Griess,† for the preparation of diazobenzol-potassium hydrate and the per cent of potassium in the compound obtained was determined. The filtrate from the milk which had been inoculated with the ferment and kept in a stoppered bottle in a warm room for ten days was neutralized with sodium carbonate, agitated with an equal volume of absolute ether, allowed to stand in a stoppered flask for 24 hours, the ether removed and allowed to evaporate from an open dish. The aqueous residue was acidified with nitric acid, then treated with an equal volume of a saturated solution of potassium hydrate and the whole concentrated on the water-bath. On being heated the mixture became yellowish-brown and emitted a peculiar aromatic odor. Both the color and odor corresponded exactly with the color and odor produced by carrying some of the artificial diazobenzol through a comparative test. On cooling, the mass crystallized, the resulting compound appearing in the test with the tyrotoxicon and in the comparative test also, in beautiful, six-sided plates, along with the prisms of potassium nitrate. The crystalline mass obtained from the tyrotoxicon was treated with absolute alcohol, filtered, the filtrate evaporated on the water bath, the residue dissolved in absolute alcohol, from which it was precipitated in colorless crystals on the addition of ether. The precipitate was collected, washed with ether, dried, and the per cent of potassium estimated as potassium sul-

*Nitrates and nitrites really give a much darker coloration than that produced with diazobenzol, and one who has made frequent observations of this test will not be likely to mistake one for the other.

†Annalen der Chemie und Pharmacie, B. 137, S. 54.

phate. .2045 gram of the substance yielded .109 gram of potassium sulphate. Per cent of potassium: calculated in, $C_6H_5N_2OK$ 24.42; found, 23.92. This analysis establishes the identity of tyrotoxicon and diazobenzol. Chemists will now appreciate the great difficulty that has been experienced in isolating the active agent of poisonous cheese. The readiness with which diazobenzol decomposes is well known. When warmed with water it breaks up into carbolic acid and nitrogen. Hydrogen sulphide decomposes it; therefore, all attempts to obtain the poison by precipitating it with some base, such as mercury or lead, and then removing the base with hydrogen sulphide, have failed. Moreover, diazobenzol is only a transition product of putrefaction. I have frequently found that leaving milk rich in the poison in an open beaker for 24 hours would be sufficient to destroy the whole of the poison.

We know nothing positively concerning the acid with which diazobenzol is combined in the milk or cheese. We prepared some diazobenzol butyrate, $C_6H_5N_2C_4H_7O_2$, and ascertained that the crystals of this compound correspond with those of tyrotoxicon, and decompose in moist air with the same rapidity.

This is the first time diazobenzol has been found as a product of putrefaction, and it is possible that many of its allied compounds may be formed in the same way.

The following experiments will show that the effects of tyrotoxicon and diazobenzol upon the lower animals are identical:—

Experiment 1. From one half gallon of some milk, which had stood in a tightly stoppered bottle for three months, there was obtained quite a concentrated aqueous solution of the poison after the spontaneous evaporation of the ether. Ten drops of this placed in the mouth of a small dog three weeks old caused within a few minutes frothing at the mouth, retching, the vomiting of frothy fluid, rapid breathing, muscular spasm over the abdomen, and after some time watery stools. The next day the dog seemed to have recovered partially; but was unable to retain any food. This condition continuing for two days, the animal was killed with chloroform. No examination of the stomach was made.

Experiment 2. Tyrotoxicon obtained from poisonous ice cream was given to a cat. Within ten minutes the cat began to retch and soon it vomited. The retching and vomiting continued for two hours, during which time the animal was under observation, and the next morning it was observed that the cat had passed several watery stools. After this, although the cat could walk about the room, it was unable to retain any food. Several times it was seen to lap a little milk, but on doing so it would immediately begin to retch and vomit. This condition continuing, after three days the animal was placed under ether, and its abdominal organs examined. We certainly expected to find marked inflammation of the stomach; but we really did find the stomach and small intestines filled with a frothy, serous fluid, such as had formed the vomited matter, and the mucous membrane very white and soft. There was not the slightest redness anywhere along the alimentary canal.

Experiment 3. Some tyrotoxicon obtained from milk which had been inoculated with poisonous cream and allowed to stand for 48 hours was administered to a large, old cat. It soon produced retching, but no vomiting or diarrhea. The amount of the poison administered in this case was small.

Experiment 4. Some tyrotoxicon from milk was given to a young, but

full grown cat. Within 15 minutes there was marked and evidently painful retching, and within half an hour, vomiting accompanied by rapid breathing. Later there were several stools, the first two of which contained fecal matter; but the subsequent ones were rice-water like and wholly free from fecal odor. After two days some more of the poison was given, and the vomiting and diarrhea again induced. The animal was then anesthetized, and examination of the stomach and intestine showed the mucous membrane blanched as was found in experiment 2.

We have the records of a number of other experiments with tyrotoxicon on the lower animals; but as the symptoms induced in all were substantially the same, it is unnecessary to note them here. We will now give the effects observed in the lower animals after the use of the prepared diazobenzol:

Experiment 5.—Gave to a large, old cat, 100 milligrams of diazobenzol butyrate. Immediately the animal began to purge. Then she lay upon the floor breathing rapidly and retching severely, for two hours, when she died. The retching was most violent, but vomiting seemed impossible. Post mortem examination showed the lungs greatly congested, but the mucous membrane of the stomach and intestine was not reddened. The stomach contained some food.

I suppose that the congestion of the lungs was due to the violent retching.

Experiment 6.—To a young but full grown Maltese cat we gave 100 milligrams of diazobenzol butyrate. With most violent retching, but without either vomiting or stool, the animal died within thirty minutes after the administration of the poison. The lungs were found acutely congested, and the stomach free from any redness. The circular fibres of the small intestine were tightly contracted.

Experiment 7.—Gave to a full grown cat 25 milligrams of diazobenzol butyrate. Within 10 minutes vomiting and purging were induced. The first stools contained fecal matter; but the subsequent ones were like rice-water and wholly free from fecal odor. After two days the cat was able to take food, then ten milligrams more of the poison was given, with the reproduction of the vomiting and purging. The animal then rapidly emaciated, and after a few days it was anesthetized and the mucous membrane of the stomach and intestine found blanched. The lungs were not congested.

Experiment 8.—10 milligrams of the poison produced profuse diarrhea, and continued vomiting in a cat.

Experiment 9.—75 milligrams produced vomiting and diarrhea, with congestion of the lungs, in a dog.

It seems unnecessary to detail any more of these experiments, as the identity of tyrotoxicon with diazobenzol is now established, not only by chemical analysis, but this proof is strengthened, if chemical analysis can be strengthened, by the action of the poison on the lower animals and by post-mortem appearance.

I think it highly probable that diazobenzol or some closely allied substance will be found in all those foods, which from putrefactive changes, produce nausea, vomiting and diarrhea. In some oysters which produced these symptoms, I have recently found tyrotoxicon.

Milk or other fluid to be tested for this poison should be kept in well stoppered bottles, for if the fluid be exposed to the air, the tyrotoxicon may decompose in a few hours. The filtrate from the milk or the filtered aqueous extract of cheese should be neutralized with sodium carbonate, then shaken

with half its volume of pure ether. Time should be given for the complete separation of the ether. Purified tyrotoxicon is insoluble in ether, and it probably owes its solubility in ether at this stage to the presence of impurities. After complete separation the ether should be removed with a pipette and allowed to evaporate spontaneously from an open dish. The residue from the ether may be dissolved in distilled water and again extracted with ether; but repeated extractions with ether are to be avoided, for as the tyrotoxicon becomes purified, it becomes less soluble in ether. To a drop of an aqueous solution of the ether residue apply the preliminary test with sulphuric and carbolic acids. To the remainder of the aqueous solution of the ether residue add an equal volume or a saturated solution of caustic potash, and evaporate the mixture on the water-bath. The double hydrate of potassium and diazobenzol will be formed if tyrotoxicon be present, and this may be recognized by its properties and reactions which have already been described.

The above mentioned experiments upon the cats and dogs, strengthen us in the belief that the development of this poison in milk is a frequent cause of cholera infantum and kindred affections. When we remember that these diseases are most prevalent among the poor classes of our large cities where fresh milk is almost unknown, we can readily understand their frequency. By such people milk is often not obtained until it has begun to sour, then it is kept at a high temperature and often in a most foul atmosphere, and we all know something of the readiness with which milk takes up bad odors. This milk is then eaten by the little ones who are weakened by poverty and everything that poverty means, insufficient food generally, and that of the poorest quality, insufficient clothing, insufficient and vitiated air. With these facts before us it is not surprising that in all our large cities thousands of children die annually from the summer diarrheas. Moreover, in our country places, how little attention is given to the food of children, we all know from actual observation. Cows stand and are milked in filthy barns and yards. The udders are generally, so far as my observation goes, not washed before the milking; the vessels for the milk are frequently found not as clean as they should be. Then there are the thousand of children that must draw their sustenance from bottles, the cleansing of which is in many families not properly attended to. Crusts of decomposing milk form around the neck of the bottle, in the tube and nipple, and lead to the rapid decomposition of the entire contents of the bottle. I think that one of the most important advantages to be secured to breast-fed children arises from the lessened danger of infection of the milk with germs which may produce poisonous ptomaines.

I would not claim that decomposed milk is the sole cause of the summer diarrheas of children, nor would I claim that tyrotoxicon is the only poison that may be developed in milk. It is only one of a large class of bodies which are produced by putrefaction, and many of these are cathartic in action.

But will this knowledge concerning the development of poisons in milk and other foods aid us in the prevention and treatment of these diseases?

Preventive measures will consist for the most part, in attention to the diet and especially to milk. I have drawn up the following rules concerning the care of milk:

1. The cows should be healthy, and the milk of any animal which seems indisposed should not be mixed with that from the perfectly healthy animals.

2. Cows must not be fed upon swill, or the refuse of breweries, or glucose factories, or any other fermented food.

3. Cows must not be allowed to drink stagnant water; but must have free access to pure, fresh water.

4. Cows must not be heated or worried before being milked.

5. The pastures must be free from noxious weeds, and the barn and yard must be kept clean.

6. The udders should be washed, if at all dirty, before milking.

7. The milk must be at once thoroughly cooled. This is best done by placing the milk can in a tank of cold spring water or ice-water. the water being of the same depth as the milk in the can. It would be well if the water in the tank could be kept flowing; indeed, this will be necessary unless ice-water is used. The tank should be thoroughly cleaned every day to prevent bad odors. The can should remain uncovered during the cooling, and the milk should be gently stirred. The temperature should be reduced to 60° F. within an hour. The can should remain in the cold water until ready for delivery.

8. In summer when ready for delivery the top should be placed on the can and a cloth wet in cold water should be spread over the can, or refrigerator cans may be used. At no season should the milk be frozen; but no buyer should receive milk which has a temperature higher than 65° F.

9. After the milk has been received by the consumer, it should be kept in a perfectly clean place, free from dust, at a temperature not exceeding 60° F. Milk should not be allowed to stand uncovered, even for a short time, in sleeping or living rooms. In many of the better houses in the country and village and occasionally in the cities, the drain from the refrigerator leads into a cesspool or kitchen-drain. This is highly dangerous; there should be no connection between the refrigerator and any receptacle of filth.

10. The only vesels in which milk should be kept are tin, glass, or porcelain. After using the vessel, it should be scalded and then, if possible, exposed to the air.

With the attention, demanded by these rules, given to milk, it will become more valuable as a food and the development of poisons in it before its introduction into the body will certainly be prevented.

But in the prevention of the summer diarrheas, attention to the food must not stop with its introduction into the body. The ferment which produces tyrotoxicon is widely distributed and it only awaits conditions suitable for its development. We do not know exactly what germ it is that produces this poison; but it is either the butyric acid ferment or some ferment which is frequently developed along with the bacillus butyricus; because I have found that if some butyric acid ferment be prepared according to the method usually followed in making butyric acid and milk be inoculated with this and allowed to stand at the temperature of the body for a few hours or at the ordinary temperature of the room for several days, the poison will appear. Moreover, as is well known the bacillus butyricus grows best in the absence of air, we have already seen that the exclusion of air favors the development of tyrotoxicon. We are aware of the fact that the butyric acid ferment frequently does develop in the stomach. Therefore, I think that the prevention of these diseases necessitates some attention to digestion. If the food lies in the stomach or intestine undigested, putrefactive changes will occur there.

During the hot months, children who are allowed to take food at will often drink large quantities of milk simply for the purpose of quenching thirst. Especially is this true when the parent forgets that a child would sometimes relish a drink of good water. I feel that this overloading the stomach with milk caused by thirst often is of no little detriment. It is hardly necessary to specify in regard to other ways in which attention should be given to the digestive organs of children. Those that partake of other foods with their milk should be allowed only the most wholesome articles, and these should be in perfect condition. Moreover, the depressing effects of extreme heat on the nervous system and its consequent injury to digestion should always be borne in mind.

Now we come to the discussion of the curative treatment of these diseases. The first thing to do is to stop the administration of milk in any form. The ferment is present in the alimentary canal, and giving the best of milk would simply be supplying the germ with material for the production of the poison. This no-milk treatment is not by any means a new idea. It has been taught for some years by a few of the best authorities; but it has not been sufficiently insisted upon. Moreover, the reason for it has not been hitherto understood. It was believed in somewhat of a vague way that the digestive organs lose their capability of digesting milk, and experience showed that the exclusion of milk led to improved results. But now that we know that a powerful poison is formed from the putrefaction of the milk, the necessity of its exclusion must become apparent to all. I reported last year a case which is so applicable here that I must be pardoned for quoting it in full. If the child had been an animal upon which I wished to experiment I could hardly have selected conditions more favorable:

"July 30, 1886, about one o'clock P. M., I was called to see the seven months' old babe of Mr. B. I found that the child had been vomiting quite constantly for some three hours. It had also passed watery stools some six or seven times. The eyes were sunken, skin cold and clammy, and pulse rapid and small. I diagnosed cholera infantum. During the preceding night the child had seemed as well as usual, and had taken nourishment freely from the mother's breast. Early in the morning it had been given a bottle of cow's milk, and soon thereafter the nausea and vomiting began. Later, as stated above, the child began to purge. The mother, furnishing an insufficient supply of milk, it had been the habit to give the child cow's milk several times through the day. I prohibited the further use of milk, both that from the mother and from the bottle, and substituted meat preparations and rice-water as foods. I also prescribed pepsin, bismuth subnitrate, chalk mixture, and camphorated tincture of opium.

"The cow's milk which had been furnished the child was from an animal kept by one of the neighbors. On the evening of the same day that the child was taken sick I obtained two quarts of the morning's milk of this animal. The milk had the appearance of very rich cream, being of a yellow tint throughout. This milk was allowed to stand through the night of the 30th in the ice-box of a refrigerator. On the morning of the 31st I began the analysis. After pouring the milk from the pitcher there remained in that vessel about two ounces of a fluid the color of port wine. Microscopical examination of this fluid showed the presence of pus and blood corpuscles. The blood was also detected by obtaining the characteristic bands of oxyhæmoglobine with the spectroscope. The milk, which had already coagulated,

was filtered. The strongly acid filtrate was rendered feebly alkaline with potassium hydrate and then agitated with absolute ether. After separation the ether was removed with a pipette and allowed to evaporate spontaneously. This residue was dissolved in distilled water and again agitated with ether. This ethereal solution left, after spontaneous evaporation, a residue which had a slightly brownish tint. I did not obtain the crystals of tyrotoxicon, doubtless owing to this trace of impurity; but the residue had the color and taste of tyrotoxicon. This residue dissolved in some distilled water and given to a cat produced retching and vomiting.

"That tyrotoxicon was present in the milk taken by the child shortly before the beginning of its illness there could now be no doubt. It is true that the milk was abnormal in other respects, also, inasmuch as it contained pus and blood.

"After the withdrawal of all milk and the use of the medicinal agents mentioned above, the child began to improve, and by the afternoon of August 1 it seemed so well that it was allowed a bottle of good cow's milk (from another animal); but soon after taking this milk it again began to vomit and purge. Milk was again withheld and the same medicinal treatment resorted to. This attack was slight, and after it the child continued to improve until the night of August 4, when the grandmother, 'who knew more about raising babies than the doctor did,' fed the child bountifully upon milk. Again the vomiting and purging began, and it was more than a week before all symptoms of gastro-intestinal irritation had disappeared. About the 15th of August milk was again allowed, at first in small quantity, and this seeming to have no harmful effect, more liberal quantities were given. The child has continued well since."

That my experience in this is not unique will be made evident by the following quotation from a recent paper by Dr. L. Emmet Holt, physician to the New York Infant Asylum, who writes as follows: "In children under two years of age not fed at the breast, it is better to withhold milk entirely. This has been a subject of careful investigation during the past summer at the New York Infant Asylum, and both the resident physicians and myself have had this proved to our satisfaction by a large number of cases. Peptonized milk is very much less likely to disagree than either condensed milk or fresh cow's milk. But in many, even this caused an aggravation in the intestinal symptoms, particularly in severe and protracted cases. Again and again have I seen relapses brought on when milk was added to the diet in cases where the stools had been practically normal for two or three days."

The food used may consist of chicken and mutton broths, beef juice, and rice or barley water. With this list, no difficulty will be experienced in giving the child sufficient nourishment. In the medicinal treatment the first thing to do is to cleanse the alimentary tract as thoroughly as possible. In the first stages of the disease there is no better agent for this purpose than castor oil. But if there have already been several serous discharges, copious enemata of water will be more suitable. These injections may contain either an astringent or a germicide, or both. For the latter, Holt recommends benzoate or salicylate of sodium, and for the former nitrate of silver or tannic acid.

The next thing to be done is to arrest the growth of the germ. This germ has been found so far to develop only in acid media. Therefore, I think it wise to administer some antacid. Probably there is nothing better in this

line than the old chalk mixture. In the preparation of the chalk mixture, the druggist should be requested to use glycerine, as many druggists still use syrup in this preparation. The presence of the sugar leads to rapid decomposition during hot weather. It has been said that the use of the antacid is irrational, because the discharges are often alkaline. Of course, the serous discharges are often alkaline, because they consist of blood serum, and will be alkaline unless they have remained in the intestine long enough to ferment; but the reaction of such discharges does not prove that the contents of the stomach and small intestine are alkaline.

As to the use of germicides, much is yet doubtless to be learned. No doubt the chief effect of subnitrate of bismuth in this disease may be due to its effect upon the germ. Holt makes an excellent showing for the salicylate of sodium, but since he has been using this drug, he has followed the no-milk diet, and doubtless his lessened mortality has been due to the exclusion of milk quite as much as to the salicylate. He uses this drug in doses of from one to three grains every two hours.

I am now making some experiments with the object of ascertaining the effect of certain germicides on the development of this poison. The results, I will give in some future paper, but I may state here what my success has been in a few experiments with mercuric chloride. The method of conducting the experiments was as follows: Four ounce bottles were filled with milk, milk and ferment, and milk and ferment with mercuric chloride, closed with glass stoppers and kept in an air bath at the temperature of the body for six hours. Then the milk was tested for tyrotoxicon with the following results :

No. 1. Bottle containing pure milk only. Result, no poison.

No. 2. Bottle containing pure milk only. Result, no poison.

No. 3. Bottle containing milk and ferment. Result, the poison present.

No. 4. Bottle containing milk and ferment. Result, the poison present.

No. 5. Bottle containing milk, ferment, and one-hundredth grain mercuric chloride. Result, poison present.

No. 6. Bottle containing milk, ferment, and one-fiftieth grain mercuric chloride. Result, poison present.